ABSTRACT

METHODS OF PROTEIN DESTABILIZATION AND USES THEREOF

This invention is directed towards methods of destabilizing proteins in living cells, and their use for the development of novel assays. In one embodiment, the invention comprises the use of non-cleavable multimerized ubiquitin fusion proteins to destabilize a target protein, such as a reporter moiety. In one aspect of this method the constructs also comprises a linker that operatively couples the reporter moiety to the multimerized ubiquitin fusion protein. In this embodiment, enzymatic modification of the linker results in a modulation of the coupling of the reporter protein to the multimerized ubiquitin domains resulting in a change in the stability of the reporter moiety. The level of the reporter moiety in the cell can then be used as a measure of the enzymatic activity in the cell. In another embodiment the invention provides for a generalized way of coordinately regulating the cellular concentration of a plurality of target proteins. In one aspect of this method, the target proteins are operatively coupled to a ubiquitin fusion protein via a linker containing a protease cleavage site. Cleavage of the linker by a protease results in uncoupling of the target protein from the multimerized ubiquitin construct, and results in an increase in the stability and concentration of the target protein.